

Original Article

Maintaining A Physiological Blood Glucose Level with ‘Glucoselevel’, A Combination of Four Anti-Diabetes Plants Used in the Traditional Arab Herbal Medicine

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Safety and anti-diabetic effects of Glucoselevel, a mixture of dry extract of leaves of the *Juglans regia* L, *Olea europea* L, *Urtica dioica* L and *Atriplex halimus* L were evaluated using *in vivo* and *in vitro* test systems. No sign of toxic effects (using LDH assay) were seen in cultured human fibroblasts treated with increasing concentrations of Glucoselevel. Similar observations were seen *in vivo* studies using rats (LD50: 25 g/kg). Anti-diabetic effects were evidenced by the augmentation of glucose uptake by yeast cells (2-folds higher) and by inhibition of glucose intestinal absorption (~49%) in a rat gut-segment. Furthermore, treatment with Glucoselevel of Streptozotocin-induced diabetic rats for 2–3 weeks showed a significant reduction in glucose levels [above 400 ± 50 mg/dl to 210 ± 22 mg/dl ($P < 0.001$)] and significantly improved sugar uptake during the glucose tolerance test, compared with positive control. In addition, glucose levels were tested in sixteen human volunteers, with the recent onset of type 2 diabetes mellitus, who received Glucoselevel tablets 1×3 daily for a period of 4 weeks. Within the first week of Glucoselevel consumption, baseline glucose levels were significantly reduced from 290 ± 40 to 210 ± 20 mg/dl. At baseline, a subgroup of eleven of these subjects had glucose levels below 300 mg% and the other subgroup had levels ≥ 300 mg%. Clinically acceptable glucose levels were achieved during the 2–3 weeks of therapy in the former subgroup and during the 4th week of therapy in the latter subgroup. No side effect was reported. In addition, a significant reduction in hemoglobin A1C values (8.2 ± 1.03 to 6.9 ± 0.94) was found in six patients treated with Glucoselevel. Results demonstrate safety, tolerability and efficacy of herbal combinations of four plants that seem to act differently but synergistically to regulate glucose-homeostasis.

Keywords: Arab herbal medicine – CAM – glucoselevel – hyperglycemia – medicinal plants

Introduction

Diabetes is a predominant public health concern that has increased steadily worldwide (1,2). The disease causes

substantial morbidity, mortality and long-term complications and remains an important risk factor for cardiovascular disease. With increasing rates of childhood and adult obesity, diabetes is likely to become even more prevalent over the coming decade. There are two types of diabetes: type I and type II. Type I juvenile diabetes is an auto-immune disease resulting in extensive destruction of the beta cells in the pancreas (3), while the cause of type

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II adult-onset diabetes remains poorly understood. All patients with type I diabetes and part of type II diabetes require daily insulin shots, replacement of the destroyed beta cell mass via islets or transplant of pancreas in order to survive (4).

Diabetes mellitus with retinopathic, neuropathic or nephropathic complications is a common debilitating and often life-threatening disease that constitutes a significant risk factor in atherosclerosis. It is well known that diabetes mellitus is associated with an increased production of reactive oxygen species and a reduction in antioxidative defences. The diabetic induced oxidative stress is pathogenetically important in diabetic complications (7). Antioxidant micro-nutrients may thus significantly contribute to reduction or prevention of diabetic complications and atherosclerosis (5–7).

The use of herbal remedies has been on the rise worldwide (7,9–11). Glucolevel, a mixture of four herbs, was developed according to the extensive herbal knowledge of the Greek–Arab medical system. A system developed over millennia, it consists of a sophisticated and highly documented tradition, which is the basis of modern European herbalism and medicine. Some of the top selling herbal products of today such as garlic, milk thistle and feverfew are derived from this system. Since ancient times diabetes has been recognized and its main symptoms were known by increased thirst, frequent urination and tiredness experienced by the diabetics.

Arab physicians and practitioners used series of plants for treating these combined symptoms (named Zarab) beside several instructions for consumption of specific food and mild practices (12,13). The traditional Arabic–Islamic medicine is still practiced and is greatly under exploited, though it may provide effective new concepts and a rich source of active herbal compounds. Two extensive surveys recently carried out among practitioners of Arabic medicine in the Middle East (8,9,14,16), have disclosed 129 plant species that are still in common use. Among them, 26 plant species are used for treating people with diabetes mellitus. *Juglans regia* L, *Atriplex halimus* L, *Olea europea* L and *Urtica dioica* L; they were part of the strongly recommended medicinal plant list (8,9). The safety of these four plants used in the present study is evident by their use in folk medicine through time. The tannins and phenolics found in walnut leaves have been acknowledged in treating high blood sugar both in Europe (10,11) and in the Middle East (8,9).

Recent studies have emphasized the use of such extracts as antioxidants in treating raised LDL levels both in Scandinavia (17) and Japan (18). The safety of salt bush is evident in Middle Eastern ethnobotany, reporting its use for heart disease and hyperglycemia (8,9). It is also widely used as animal food in Europe (19,20) and is of

high economic value in Sicily (21) suggesting that salt bush is not toxic for mammals. Chromium and magnesium salts obtained using salt bush have been evidenced to inhibit or prevent diabetes in sand rats (22,23). The safety of olive leaves has been widely documented both in Europe (23–27) and the Middle East (8) as in the case of nettle in Europe (23,26–28), in Asia (9,29) and in the United States where nettle has a GRAS (Generally Recognised As Safe) status (23).

To consider anti-diabetic plants for clinical use, the ideal plant should to possess the following properties: Traditionally it has been used in more than one country, has experimentally documented constituents, has hypoglycemic activity, low toxicity and botanical abundance (23). We hypothesized that the extracts from the four plants when combined may disclose synergistic effects on different levels of glucose-insulin homeostasis thus adding to therapeutic efficacy. This study was aimed at investigating safety and efficacy of a fixed mixture of these plants. As for safety, we performed *in vitro* and animal studies. As for therapeutic efficacy, we carried out controlled studies in animals and an open human investigation.

Methods

Preparation of Glucolevel

The leaves of walnut, olive, nettle and salt bush were collected, dried under shade, cleaned and sterilized by steam for 2 h and powdered to a fine grade as extracts. Therefore, 10 kg milled plants were extracted with 30 g food grade 50% ethyl alcohol for 2.5 hours at a 70 °C in a stainless steel tank and filtered through a batch centrifuge. The residues were extracted with another 30 kg, 50% ethyl alcohol and filtered through the batch centrifuge. The filtrates from both extractions one and two were mixed and evaporated. The yield of our extraction was about 10%. Glucolevel tablets each of 510 mg net weight were produced by adding 7 mg Vitamin C and 150 mg tricalcium phosphate to 353 mg of dried plants. The yield of this extraction was also about 10%. Glucolevel concentrations used in the present study were determined according to traditional uses, where total amount in capsules given for each diabetic patient/day is equivalent to 7–10 gm dried plant leaves.

Analyses for Safety

Lactate Dehydrogenase

In the lactate dehydrogenase (LDH) assay, the leakage of the cytoplasmic located enzyme LDH into the extracellular medium was measured. Presence of the

exclusively cytosolic enzyme, LDH, in the cell culture medium is indicative of cell membrane damage (45). For the LDH assay, 1.5×10^4 cells from the fibroblasts cell line 3T3 were seeded per well of 96-microtiter plates. Twenty-four hours after cell seeding, cells were treated with two concentrations of Glucolevel (180 and 360 mg/ml) varying. After 24, 48 and 72 h of treatment, the supernatants were collected from each well. Cell monolayers were then treated with a cell lysis solution for 30 min at room temperature. LDH activity was measured in both the supernatants and the cell lysate fractions by using CytoTox 96, a non-radioactive cytotoxicity assay kit (Promega, WI, USA), in accordance with the manufacturer's instruction. The intensity of the color is proportional to LDH activity. The absorbance was determined at 490 nm with a 96-well plate ELISA reader. The percent of LDH release from the cells was determined using the formula: LDH release = (absorbance of the supernatant)/(absorbance of the supernatant and cell lysate)*100.

LD50

32 Male Spague–Dawley rats (average weight: 161 ± 17 g) were divided into four groups. Increasing large single doses of Glucolevel (up to 50 g/Kg) were placed directly into the stomach of each group and were observed for 14 days to determine the LD50.

In-vitro Efficacy

Glucose uptake by yeast cells

Glucose transport through yeast cell membrane is based upon the Warburg method of (1946), which is a fermentation process. The amount of CO₂ produced was measured in milliliters during anaerobic control conditions and during the addition of Glucolevel extract, (2 mg/ml) and taken to reflect the extent of glucose-entrance into yeast cells (30).

Glucose Intestinal Absorption (inverted intestine sac test)

The upper part of the small intestine was obtained from Sprague Dawley male rats (average weight: 161 ± 17 g), washed with physiologic solution and incubated in Krebs solution. The inner surface of the gut segment was inverted with the help of the Pasteur Pipette. Then the bottom part of the inverted segment was tied before filling it with Krebs solution and placed in Erlenmeyer containing 7 ml of 1% starch solution and 1 ml of 1% pancreatin solution. The apparatus was bubbled with gas composed of 95% O₂ and 5% CO₂. The glucose amount obtained outside the inverted intestine was taken to represent starch digestion, whereas the glucose amount inside the sac was taken to represent glucose absorption in the gut. Determination of glucose values was done

using the DNS method (31,32). To avoid variations between inverted intestine segment weights, protein concentrations were determined at the final stage with the Lowry method (33) and segment weights were adjusted. Tests were performed at baseline and during the addition of various Glucolevel concentrations.

Efficacy in Rats

Diabetes mellitus was induced in 14 rats by a single intraperitoneal injection of 80 mg Streptozotocin/kg in sterile 0.1 M citrate buffer (pH 4.5). Diabetes mellitus was developed gradually in them, as assessed by blood glucose levels, which reached about 400 mg within one week. These rats were treated with 150 mg Glucolevel extract/kg/day for 3 weeks. An oral glucose tolerance test was performed on three groups of rats with a glucose load of 3 g/kg. Diabetic rats were divided into two groups of seven rats each during glucose tolerance testing and one group only received Glucolevel 150 mg/kg together with the glucose load. The same glucose load was given to 6 non-diabetic rats the third group.

Clinical Investigations: Selection of Volunteers

Human volunteers were selected on the basis of type II diabetes mellitus of recent onset, i.e. they were only on diet therapy and not on treatment with hypoglycemic pharmacologic agents. They were recruited from four General-Physician-Clinics in the Galilee region (Israel) and were motivated to take herbal therapy. After a thorough review of Glucolevel-components, they were asked to continue their daily activities; leave diet habits unchanged and take one tablet of Glucolevel 3 times daily. They were also asked to restrain from consuming any medications during the study period of 4 weeks. An informed consent was obtained from each subject who was given a box containing 90 tablets of Glucolevel.

Clinical Protocol

At baseline, glucose blood levels were estimated in each subject who was scheduled for the next visit in 1 week and was asked to return the Glucolevel box so that returned tablets could be counted as the only measure of patient compliance with the protocol. This was repeated during each of the three consequent visits when glucose levels were estimated and careful investigations of well-being as well as any adverse effects were undertaken.

Statistics

The Wilcoxon signed rank was used. Comparison between groups was performed by the Wilcoxon

rank-sum test. A 0.05 level of significance was set. Data obtained were expressed as mean ± standard error of mean (SEM).

Results

Safety

The safety of Glucolevel was evaluated both *in vitro* using LDH assay on cultured fibroblasts and *in vivo* by measuring the LD50 in rats. Fig. 1 shows LDH-release from 3T3 cells treated for 24h (Fig. 1A), 48h (Fig. 1B), and 72h (Fig. 1C) with 180 or 360 mg Glucolevel/ml. Compared to untreated control cells, no significant change in LDH-release was observed whether as a function of increasing the concentrations or as a function of an increased incubation period. Similar observations were observed *in vivo* by measuring the LD₅₀ in rats. Glucolevel showed an LD₅₀ by concentration of about 25 g/kg.

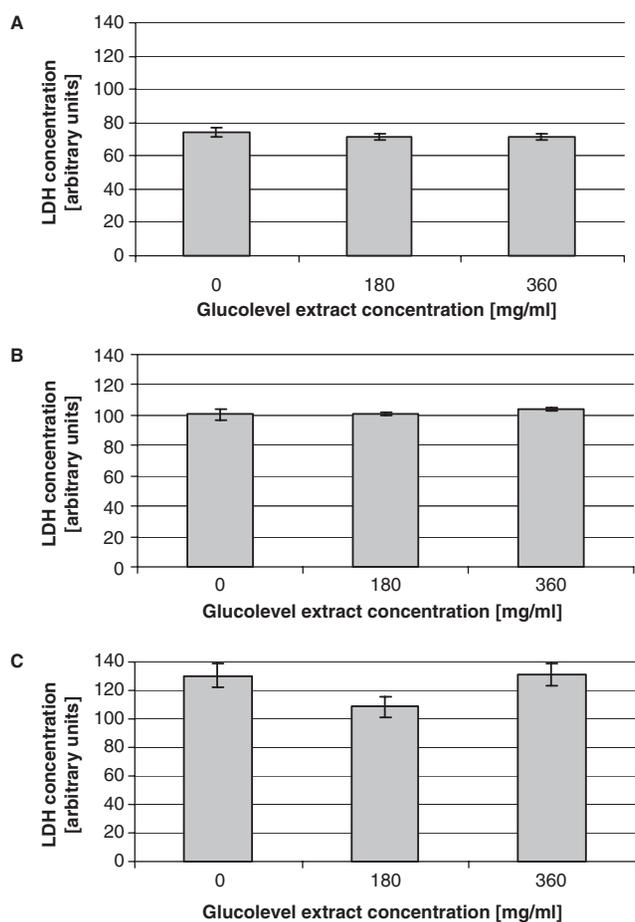


Figure 1. LDH-release in arbitrary units from cultured human fibroblasts. Cells were treated with two concentrations of Glucolevel (180 and 360 mg/ml) for 24h (A), 48h (B) and 72h (C). Data shown represents the mean + SEM from three independent experiments carried out in triplicates.

Efficacy

Glucose Uptake

Fig. 2 shows the amount of CO₂ produced within 1 h of fermentation in yeast cells under controlled conditions with the presence/absence of 2 mg/ml of Glucolevel. In this assay, CO₂ is a marker for glucose inside yeast cells, the more CO₂ is produced the more glucose enters into the yeast cells. As seen in Fig. 2, glucolevel significantly increased the glucose uptake through the yeast plasma membrane. The glucose level was two-fold higher after Glucolevel treatment, compared with the control group.

Glucose Transport through Inverted Intestine

Table 1 shows results of glucose measurements inside and outside the inverted intestine at baseline and during the addition of 10, 20 and 30 mg glucolevel/ml. Glucolevel at concentrations of 20 and 30 mg/ml significantly decreased the glucose values inside the inverted intestine ($P < 0.01$), whereas, slight but insignificant reductions of these values were obtained outside the inverted intestine.

Glucolevel Reducing Glucose Levels in Streptozotocin-induced Diabetic Rats

Induction of diabetes mellitus by Streptozotocin was confirmed by measuring the glucose levels. Within 1 week,

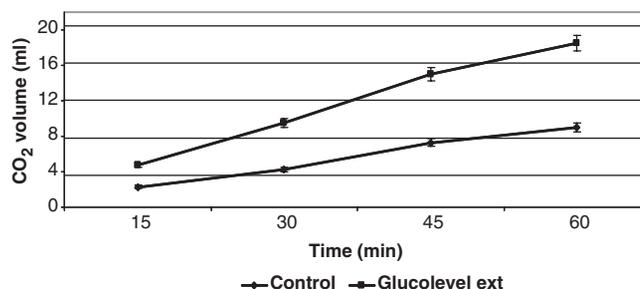


Figure 2. The amount (ml) of CO₂ produced within 1 h of fermentation of yeast cells under control conditions (lower curve) and during the addition of 2 mg Glucolevel/ml (upper curve).

Table 1. Glucose values measured inside and outside the inverted intestine sac placed in starch-pancreatin solution at control conditions (0) and during the addition of different concentrations of the Glucolevel extract

Glucolevel (mg/ml)	Glucose values mg/ml protein inside the sac	Glucose values mg/ml protein outside the sac
0	2.9 ± 0.2	28 ± 2
10	2.7 ± 0.3	27 ± 3
20	1.6 ± 0.3*	25 ± 5
30	1.5 ± 0.1*	24 ± 4

* $P < 0.01$ versus control conditions (0).

glucose levels reached a level of 400 ± 50 mg/dl in the 14 rats. Treatment with Glucollevel for three weeks significantly reduced glucose levels in these rats to 210 ± 22 mg/dl ($P < 0.001$). Fig. 3 summarizes glucose tolerance testing in these rats with and without the addition of Glucollevel 150 mg/kg and compares the results with those obtained in six non-diabetic rats during the same testing. Non-diabetic rats exhibit a normal glucose tolerance curve (lower curve). Diabetic rats with Glucollevel added to the glucose load, and they disclose a near normal curve (middle curve) with the 2–3 h glucose levels of 180 ± 10 mg%, which is significantly ($P < 0.01$) lower than the levels (290 ± 30 mg/dl) disclosed in diabetic rats without additional Glucollevel (upper curve). These levels in the latter group are still above baseline levels.

Clinical Analyses

The 16 test persons recruited for the study had an age range of 48–67 years and their compliance with the study protocol was excellent. No test person took any pharmacologic drug during the study period. No minor

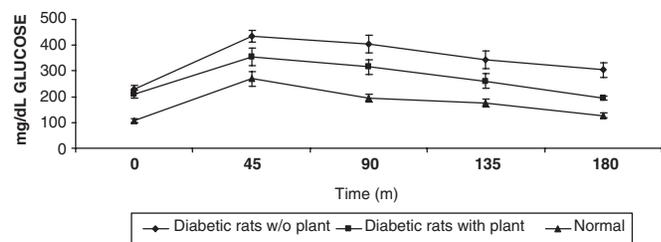


Figure 3. Glucose tolerance testing in normal rats (lower curve) and in two groups of diabetic rats treated with Glucollevel for 3 weeks (upper two curves). The middle curve was obtained from one diabetic group to which the same extract was added to the glucose load during testing.

or major adverse effect was noted and the Glucollevel was well tolerated by all subjects. During the first week of Glucollevel consumption, baseline glucose levels were reduced from 290 ± 40 mg/dl to 210 ± 20 mg/dl in these subjects ($P < 0.001$). According to baseline glucose levels, a subgroup of 11 had glucose levels below 300 mg/dl and the other subgroup had levels ≥ 300 mg%. As shown in Fig. 4, the former subgroup achieved clinically acceptable glucose levels during the 2–3 week of Glucollevel consumption while the latter group needed 1 week more to achieve clinically acceptable glucose levels.

Table 2 shows the effects of glucollevel on the glucose level and hemoglobin A1C (HbA1C) in six diabetic patients, who did not fully respond to conventional

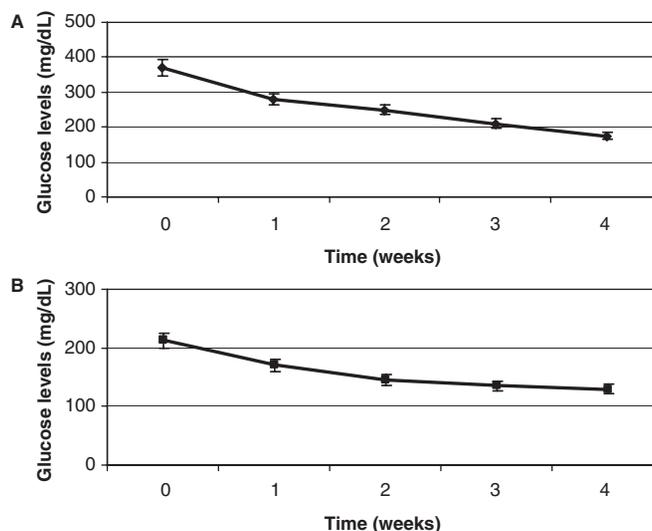


Figure 4. Glucose levels during a 4 week period of Glucollevel consumption 1 tablet \times 3 daily in those subjects with baseline glucose levels below 300 mg/dl (lower curve) and in those with baseline glucose levels ≥ 300 mg/dl (upper curve).

Table 2. The effects of Glucollevel on blood glucose levels and HbA1C values with and without conventional anti-diabetes drugs

#	Diabetes duration (Years)	Diabetes Drugs	Glucose values without Glucollevel	Glucose values with Glucollevel	HbA1C-A With Diabetes Drugs	HbA1C-B With Diabetes drugs	HbA1-C with Diabetes drugs and Glucollevel
1	7	Glucophage + Glubin	180–250	90–130	7.8	8	6.3
2	9	Glucophage + Glubin + Avandia	200–290	110–140	7.5	7.9	6.5
3	11	Insulin 45 units	200–270	120–150	9.5	8.7	7.3
4	5	Glucophage	130–150	80–120	7.9	7.7	6.7
5	6	Insulin 100 units	180–200	90–140	10	10	8.7
6	4	Glucophage + Glubin	250–200	100–120	7.9	7	6.2
Average					8.4 \pm 1.04	8.2 \pm 1.03	6.9 \pm 0.94

The tests were conducted in 22 diabetic patients who didn't fully respond to conventional therapeutics and remained with high glucose levels. The patients were allowed to continue taking their medication were the sugar levels being continually monitored and HbA1C value was determined after three months according to Saudek *et al.* (46).

HbA1C-A: Values of A1C of the patients were determined during treatments by their doctors (using only conventional drugs).

HbA1C-B: Values of A1C of the patients were determined during treatments by their doctors (using only conventional drugs) in order to make medical comparison to the treatment efficacy (drugs have to reduce A1C values).

HbA1C-C: Values of A1C after taking diabetes drugs and Glucollevel capsules.

therapeutics and remained with high glucose levels. The test aimed to clarify whether Glucolevel supplementation can contribute to reduced the glucose levels in this group. The patients were allowed to continue taking their medication during the whole test period. The sugar levels were continually monitored and HbA1C values were determined after three months. The results indicated a significant reduction in HbA1C values compared with conventional treatment and showed positive synergetic effect.

Discussion

Despite all the marvelous advancements in modern medicine, traditional herbal medicine has always been practiced. Cultural beliefs and practices often lead to self-care or home remedies in rural areas and consultation with traditional healers. Alternative therapies have been utilized by people in our region who have faith in spiritual healers. About 400–450 medicinal plants are found in the Palestinian-Israelian region and about 129 plant species are still used for treating of various human diseases, including diabetes, liver diabetes, as well as skin, respiratory, digestive and cancer diabetes, along with other diseases (14–16). It has already been mentioned that since ancient times diabetes has been recognized and its main symptoms were known by increased thirst, frequent urination and tiredness experienced by diabetics. Arab physicians and practitioners had used series of plants for treating these combined symptoms. *Juglans regia* L, *Atriplex halimus* L, *Olea Europea* L and *Urtica dioica* L were part of the strongly recommended anti-diabetic medicinal plant. Therefore, a mixture of extracts of these four plants was prepared and its efficacy and safety were evaluated in the present study.

Safety Studies of Glucolevel

Our data indicates a high level of safety in Glucolevel with very large concentrations of 25 g/kg to yield the LD50. Glucolevel at concentrations as high as 360 mg/ml did not show any sign of cellular toxicity as evidenced by LDH-release. Similar results were reported for plants used for the preparation of Glucolevel. For example, (i) in a long-term clinical study of nettle extract for benign prostatic hyperplasia (37), a daily dose of 320 mg was well tolerated and showed no evidence of adverse effects. (ii) Extracts of walnut leaves protect against cellular-toxicity and to reduce cyclophosphamide-induced biochemical toxicity (38). (iii) Several extracts of salt bush have been reported to be non-toxic to the stored plant insect (39). (iv) As for olive leaves, doses as high as 1200 mg/kg for 60 days in rats were completely atoxic (40), and aqueous extracts from these leaves were given to two groups of hypertensive patients with no side effects and with a promising clinical efficacy (41).

As experienced in good clinical practice, smaller doses of synergistic drugs may yield a better therapeutic efficacy with lower side effects. This could explain the fact that Glucolevel was well tolerated by all our subjects and no adverse effects could be traced.

Efficacy studies with Glucolevel

Anti-diabetic effects of Glucolevel were seen in (i) glucose uptake by yeast cells, (ii) inhibition of glucose intestinal-absorption in a rat gut-segment, (iii) in Streptozotocin-induced diabetic rats for 2–3 weeks and (iv) in clinical studies with sixteen human volunteers with recent onset of type 2 diabetes mellitus who received Glucolevel tablets 1 × 3 daily for a period of 4 weeks. The used Glucolevel concentrations in the present study were determined according to traditional uses, where the total amount in capsules given for each diabetic patient/day is equivalent to 7–10 gm dried plant leaves.

The main pathologic effects in diabetes mellitus consist of excessive hepatic glucose production, peripheral insulin resistance and defective β -cell secretory function. Available per patient, oral hypoglycemic agents are directed at stimulating insulin secretion (sulfonylureas), inhibiting excessive hepatic glucose production (metformin or biguanides), delaying the absorption of carbohydrates in the gut by inhibiting α -glucosidase (acarbose) or reducing insulin resistance (troglitazone) primarily in skeletal muscle, but also in adipose tissue (34). We propose that the combination of the four herbs in Glucolevel may represent a combination of the four different action sites of oral hypoglycemic drugs (34). Furthermore, chromium-magnesium salts contained in salt bush (23) and evidenced as an insulin cofactor to facilitate glucose entry into muscle and fat cells (35,36), as well as the antioxidants contained in walnut and olive leaves (17,25), may all add to the therapeutic efficacy of Glucolevel.

The efficacy of Glucolevel in diabetic animals was evident during the treatment given for 3 weeks as it reduced glucose levels from about 400 to about 200 mg/dl. As shown in Fig. 3, these diabetic animals still disclosed an abnormal glucose tolerance curve. The addition of Glucolevel to the glucose load almost normalized the glucose tolerance curve in these animals by reducing both the hyperglycemic peak and the late glucose levels. A treatment period of 2–3 weeks achieved adequate glycemic control in subjects with baseline glucose levels below 300 mg/dl. Adequate glycemic control in those with higher baseline glucose levels was achieved after an additional treatment period of 1–2 weeks more.

Possible Action Mechanism of Glucolevel

Scientific evidence obtained so far is indicating hypoglycemic and antioxidant properties of each of the four

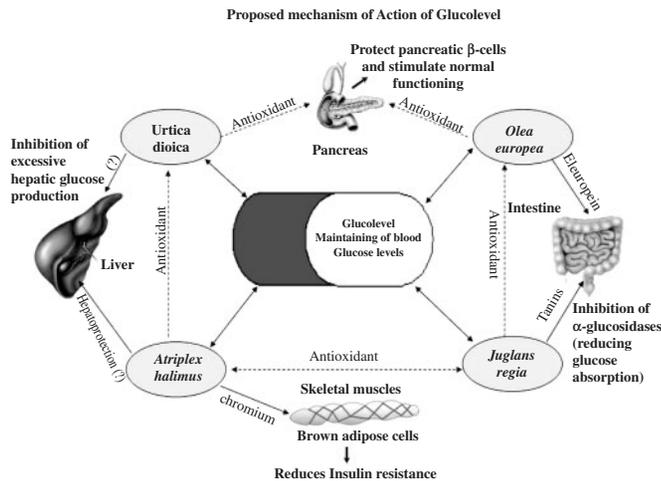


Figure 5. Proposed mechanism of Glucoclevel. Results obtained and previously published scientific evidence indicate hypoglycaemic and antioxidant properties of each of the four herbs contained in Glucoclevel. *Atriplex halimus* is extremely effective in potentiating antidiabetic through insulin which facilitates glucose entry into muscle and fat cells. The *Urtica dioica* component may decrease glucose production by the liver whereas, oleuropein and tannins in *Olea europea* and *Juglans regia* leaves may act as α -glucosidase inhibitors thus reducing the absorption of carbohydrates in the gut. The antioxidants contained in the four plants may exert hepatic-pancreatic cytoprotection and enhance the therapeutic efficacy of Glucoclevel.

herbs contained in Glucoclevel. The main active ingredient in the olive leaf was reported to be oleuropein, which disclosed a distinct hypoglycaemic effect at a dose of 16 mg/kg (42), together with hypotensive and hypolipidemic properties (40,42). Tannins and polyphenolics in walnut leaves were discovered to be potent antioxidants (17) and revealed strong scavenging activity against both superoxide and hydroxyl radicals (43). An animal-model for diabetogenesis and obesity (22) proved that salt bush is an extremely effective antidiabetic herb (22, 23) and that also shows an insulin potentiating effect (35,36). There is also the evidence that nettle extracts possess hypoglycaemic properties and improve glucose tolerance (23,45). The *in vitro* experiments disclosing that Glucoclevel facilitates glucose entry into yeast cells during anaerobic fermentation may be attributed to an effect of salt bush content in the product. The nettle component is supposed to decrease glucose production by the liver whereas; oleuropein and tannins in olive and walnut leaves are supposed to act as α -glucosidase inhibitors thus reducing the absorption of carbohydrates in the gut. Such an effect was evidenced in our experiments with the inverted intestine segment at a concentration of 20 mg/ml of the Glucoclevel extract (Fig. 5).

Conclusions

We selected four medicinal plants used in the traditional Arab herbal medicine for the preparation of Glucoclevel.

Our results obtained in the present study demonstrate safety, tolerability and efficacy of such herbal combinations of the four plants that seem to act differently but synergistically to regulate glucose-homeostasis.

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